

Evaluation of Peruvian Maize for Resistance to European Corn Borer (*Lepidoptera: Pyralidae*) Leaf Feeding and Ovipositional Preference

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ABSTRACT Partial control of the European corn borer, *Ostrinia nubilalis* (Hübner), in maize, *Zea mays* L., has been achieved through germplasm resistant to leaf feeding that was incorporated into breeding populations. Leaf-feeding resistance to European corn borer in maize had generally been attributed to presence of the chemical 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). New sources of resistance, other than that mediated by DIMBOA, are desirable for developing new resistant cultivars. From 1990 to 1992, 1,601 accessions of Peruvian maize maintained in the U.S. National Plant Germplasm System were evaluated for leaf-feeding resistance to European corn borer. Eleven resistant accessions were identified, all of which originated from Peru's north coast. The 11 resistant accessions were then analyzed for 6-methoxybenzoxazolinone (MBOA), the degradation product of DIMBOA and an indicator of DIMBOA levels present in the plant. All 11 resistant accessions contained low MBOA concentrations, equivalent to that found in the susceptible inbred WF9, indicating that DIMBOA is not the basis of this resistance. The factor(s) underlying this source of host plant resistance remains to be determined. Six morphologically diverse Peruvian maize races were evaluated in the greenhouse for ovipositional nonpreference by the European corn borer. Some differences were noted, but morphological factors could not be confirmed as causing the differences.

KEY WORDS *Ostrinia nubilalis*, host plant resistance, corn, oviposition

THE EUROPEAN CORN borer, *Ostrinia nubilalis* (Hübner), is one of the most damaging insects on maize, *Zea mays* L. (Ferriss 1988). A bivoltine strain of European corn borer, predominant throughout the northern corn belt, damages the maize plant at 2 distinct stages: the whorl stage and shortly after anthesis (Showers et al. 1989). Damage by early-instar European corn borer during the whorl stage of maize plant development is characterized by leaf feeding in the whorl of the plant. This causes physiological losses to the plant because of a reduction in leaf area (Dicke 1954).

The economic significance of the European corn borer has been reduced by the identification of resistant maize germplasm and the backcrossing of these resistant cultivars into elite maize lines. Development of maize resistant to European corn borer larval feeding has been one of the most efficient methods of control for this insect (Barry and Darrah 1991).

European corn borer leaf-feeding resistance in maize adapted for the corn belt has been attributed to the chemical 2,4-dihydroxy-7-methoxy-1,4-

benzoxazin-3-one (DIMBOA) (Klun et al. 1967). Other (for example, non-DIMBOA) sources of European corn borer resistance would give plant breeders additional genetic material to develop resistant maize breeding populations. Resistant tropical and exotic maize populations that do not rely on DIMBOA for resistance have been identified, but the factor(s) causing the resistance is as yet undetermined (Sullivan et al. 1974, Scriber et al. 1975, Chiang and Hudson 1976). The probability of finding new sources of European corn borer resistance by using a unique base of resistance may increase when the diverse maize germplasm from the Tropics is evaluated.

Peruvian maize contains a high degree of genetic variability that has been categorized into at least 42 races containing multiple genetic variants within each race (Grobman et al. 1961). In the middle to late 1980s, a large number of Peruvian maize accessions was acquired by the U.S. National Plant Germplasm System (NPGS); these are currently maintained by the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA.

We report herein the results of evaluating the NPGS maize collection from Peru to identify unique sources of resistance to European corn borer leaf feeding. The 1st objective of this study was

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to evaluate maize that is maintained at the NCRPIS for sources of leaf-feeding resistance to the European corn borer. A 2nd objective was to determine if the resistance was based on the chemical DIMBOA. A 3rd objective was to evaluate 6 Peruvian races of maize to determine if European corn borer *ovipositional nonpreference* exists within the maize collection.

Materials and Methods

Field Evaluation for Resistance to European Corn Borer Leaf Feeding. In 1990 and 1991, 1,601 Peruvian maize accessions were planted in single rows with a jab planter. Four to 5 seeds per hill were planted, with each row containing 3 hills. Rows were 1.5 m long and spaced 1.5 m apart. Each row was thinned to 6 plants after emergence. A resistant control inbred (CI31A) and a susceptible control inbred (WF9) were planted throughout the field to aid in monitoring infestation levels. Test plants were artificially infested at the V4 stage (Benson and Reetz 1985) of maize development. Approximately 300 neonate European corn borers (courtesy USDA-ARS Corn Insects Research Unit, Ames, IA) were deposited into the whorl of each plant. Larvae were applied to the plants using a "bazooka" applicator developed by Mihm (1963). Three weeks after infestation, the test plants were rated visually for European corn borer leaf-feeding damage by using a 9-class rating scale developed by Guthrie et al. (1960). With this scale, resistant accessions receive lower numeric ratings.

Maize accessions with an initial damage rating of 3 or less were retested in 1992. Fourteen Peruvian maize accessions plus a resistant (CI31A) and susceptible control (WF9) were retested by using a randomized complete block design with 8 replications. Plot mean values were used for analysis of variance (ANOVA).

Data were analyzed with the ANOVA-2 program of MSTAT-C 4.0 (MSTAT Development Team 1989). When the *F* value for treatments was significant ($P < 0.05$), means were separated with the RANGE program of MSTAT-C 4.0 with the least significant difference (LSD) test ($\alpha = 0.05$).

Determination of DIMBOA Content in Resistant Peruvian Maize Whorl-Leaf Tissue. In 1991, the resistant Peruvian maize accessions and 2 controls (CI31A, resistant and WF9, susceptible) were planted in the field. Accessions were grown in the field as a randomized complete block design with 2 replications. At the V4 maize plant stage, whorl-leaf tissue was removed, frozen, thawed, dried in a laboratory oven at 50°C for 72 h, and ground in a Wiley mill to a fine powder. MBOA was quantified by the method of Robinson et al. (1982). Ground whorl-leaf tissue (0.5 g) was extracted with 20 ml of boiling distilled water and filtered (Whatman No. 1). The residue was again extracted 3 times with ≈ 7 ml of hot water. The cooled extract was acidified to pH 1–2 with concentrated HCl,

partitioned with ethyl ether, and dried with Na_2SO_4 . The organic phase was concentrated to ≈ 40 ml and decanted from the Na_2SO_4 . The organosoluble material was evaporated to dryness.

The MBOA was isolated from each plant extract by normal phase, thin-layer chromatography (TLC) on Beckman silica gel 60 F 254 (20 by 20 by 0.25 mm) with a 1-dimensional, dual-developing system: (1) 4:4:1 CHCl_3 :ethyl acetate:cyclohexane; (2) 17:3 cyclohexane:isobutanol. Extracts were dissolved in 1:1 benzene:ethyl acetate and 100 μl applied to the plate. The relative front of MBOA was determined by cochromatography with 50 μg of MBOA standard (Sigma, St. Louis, MO).

The MBOA from each sample was eluted from the TLC with 6 ml of 95% ethanol (EtOH). MBOA was quantified photometrically (Shimadzu model UV160A, Kyoto, Japan) at 231 nm. A normal distribution was established using MBOA values from this study. A probability table was used to determine significant differences. *Z* values were used in the probability table to determine probabilities for the normal distribution. *Z* values were calculated by using the sample standard deviation (*s*), mean of the sample population, and the value in question (*x*) in the following formula $Z = (x - \text{mean})/s$.

Ovipositional Preference. Six Peruvian maize accessions representing 6 races were selected for their variations in leaf pubescence, leaf color, leaf venation, and leaf width from data presented in Grobman et al. (1961) and the USDA-ARS Germplasm Resources Information Network (GRIN; Beltsville, MD). A resistant ovipositional preference control, Ill.A (NSL-30861), and a susceptible ovipositional preference control, Ia.L317 (NSL-65973), were chosen from data presented in Everly et al. (1979). Seven maize plants from each of the 6 accessions and the 2 controls were grown in 24-cm-diameter pots in the greenhouse.

Ovipositional selection of the maize accessions was conducted within a cage consisting of six 1.5-m² panels with a 2.5-cm² wooden frame covered with lumite screening (Chicopee Manufacturing, Chicopee, GA). The 6 panels were assembled in the greenhouse to form a 1.5-m³ enclosed cage.

At the V4 stage, 1 plant from each of the 6 accessions and 1 plant from each control were placed randomly within each cage. Photoperiod was maintained at 12:12 (L:D) h and relative humidity at 70%. Thirty newly mated European corn borer females were released into each cage. After 6 d, the adults were removed from the cages and the number and location of egg masses on each plant were recorded. The experiment was conducted as a randomized complete block design with 7 replications. Data were analyzed with MSTAT-C 4.0 (MSTAT Development Team 1989). When the *F* value for treatments was significant ($P < 0.05$), means were separated with the RANGE program of MSTAT-C 4.0 by using the least LSD test ($\alpha = 0.05$).

Table 1. Number of accessions receiving ratings in each rating category from 1990 and 1991 field tests conducted at Ames, IA.

| Rating category | No. accessions |
|-----------------|----------------|
| 1 | 1 |
| 2 | 2 |
| 3 | 11 |
| 4 | 79 |
| 5 | 195 |
| 6 | 266 |
| 7 | 381 |
| 8 | 409 |
| 9 | 257 |
| Total | 1,601 |

Results

Leaf Feeding. Table 1 presents the rating distributions from the 1,601 accessions evaluated in 1990 and 1991. The ANOVA for the 14 Peruvian maize accessions retested in 1992 showed highly significant differences among accessions ($F = 16.02$; $df = 15, 105$; $P < 0.01$, Table 2). The susceptible inbred control WF9 had the highest rating, and accessions 503723 and 503731 were not significantly different from the resistant control, CI31A (Table 2). Eleven of the 14 accessions retested rated ≤ 3.2 and were considered resistant (Table 2).

Quantification of MBOA varied from 0.144 mg/g in accession 503764 to 1.104 mg/g of dried whorl tissue in CI31A (Table 3). The mean of the sample population was 0.292 mg MBOA/g of dried whorl tissue, and the sample standard deviation was 0.251 in the European corn borer-resistant Peruvian maize. Levels of MBOA were not significantly different from the mean MBOA level in the susceptible control inbred WF9.

Table 2. European corn borer leaf-feeding ratings for 14 Peruvian maize accessions selected for retesting in 1992 at Ames, IA.

| Accession no. | Mean leaf-feeding ratings ^{a, b} | Peruvian maize race |
|---------------|---|---------------------|
| WF9 | 7.1a | Susceptible check |
| 220065 | 5.9b | LaMolina hybrid |
| 571506 | 4.2c | Piscorunto |
| Ames-13930 | 3.9cd | Tuxpeño |
| 503725 | 3.2de | Mochero |
| 503806 | 3.2de | Alazan |
| 503849 | 3.2de | Alazan |
| 503764 | 3.0def | Mochero |
| Ames-10623 | 3.0def | Arizona |
| 503720 | 2.9def | Mochero |
| 503722 | 2.9def | Mochero |
| 503728 | 2.9def | Mochero |
| 503727 | 2.8ef | Mochero |
| 503723 | 2.6efg | Mochero |
| 503731 | 2.2fg | Mochero |
| CI31A | 1.8g | Resistant check |

Means followed by the same letter are not significantly different according to the LSD test ($P = 0.05$).

^a Guthrie et al. (1960) 1-9 rating scale.

^b Values represent the mean of 8 replications.

Table 3. Mean levels of MBOA and Z scores for 11 Peruvian and 2 check maize samples at the mid-whorl stage at Ames, IA, in 1992.

| Accession no. | MBOA concn mg MBOA/g tissue | Z value ^a |
|---------------|--------------------------------|----------------------|
| CI31A | 1.104 | 3.23 |
| 503720 | 0.377 | 0.34 |
| 503727 | 0.276 | 0.06 |
| 503722 | 0.274 | 0.07 |
| Ames-10623 | 0.232 | 0.24 |
| WF9 | 0.217 | 0.30 |
| 503723 | 0.214 | 0.31 |
| 503725 | 0.210 | 0.33 |
| 503849 | 0.208 | 0.33 |
| 503731 | 0.199 | 0.37 |
| 503806 | 0.175 | 0.47 |
| 503728 | 0.165 | 0.51 |
| 503764 | 0.144 | 0.59 |

^a Z value equals the number of standard deviations from the sample mean.

Ovipositional Preference. There was a highly significant difference ($F = 5.41$; $df 7, 42$; $P < 0.01$) between accessions for total number of egg masses oviposited. The number of egg masses oviposited on the susceptible control (NSL-65873) and the resistant control (NSL-30861) were not significantly different (Table 4). Accessions 503796 and 485330 had significantly fewer oviposited egg masses than 485320, 571506, and 515036 (Table 4).

Discussion

The 11 resistant Peruvian maize accessions identified in this study belong to 3 different races of maize as defined by Crobinan et al. (1961). Eight accessions are race Mochero, an early, drought-resistant maize with a floury kernel type. Two accessions are race Alazan, another floury kernel type maize, which is also drought resistant. One accession was race Arizona, which is an introduced Tuxpeño and has a dent kernel type (Table 2). All 11 accessions originated from irrigated valleys on Peru's north coast, and all are resistant to European corn borer leaf feeding even though the European corn borer has never existed in Peru.

Table 4. Number of egg masses deposited by European corn borer during an ovipositional preference test in the greenhouse at Ames, IA, in 1992.

| Accession no. | Mean ^a |
|---------------|-------------------|
| 515036 | 16.3a |
| 571506 | 15.1a |
| 485320 | 14.7a |
| 485347 | 10.9ab |
| 503796 | 6.9bc |
| 485330 | 6.0bc |
| NSL-65873 | 2.6c |
| NSL-30861 | 1.7c |

Means followed by the same letter are not significantly different according to the LSD test ($P = 0.05$).

^a Means are calculated from 7 replications.

One accession, 571506 (maize race Piscorunto; a high-elevation maize from southern Peru with a floury kernel type), received a resistant rating of 1 during the initial screening (Table 1). After retesting 571506 in 1992, considerable variation was found in plant expression of resistance to European corn borer leaf feeding. Some plants were heavily infested; however, 1 or 2 plants in each row were highly resistant. Thus, a source of high-altitude maize with European corn borer leaf feeding resistance may exist in some genotypes within this accession.

Significantly lower levels of MBOA were found in the resistant Peruvian maize accessions than in the resistant control CI31A. Levels of MBOA in these accessions were not significantly different from the levels in the susceptible control WF9. This indicates that DIMBOA concentration is not responsible for the resistance of these Peruvian maize accessions to European corn borer leaf feeding. A non-DIMBOA basis of resistance is suggested.

Non-DIMBOA resistance to leaf feeding by European corn borer may be useful for developing highly resistant maize that combines 2 or more resistance bases; resistant maize that could be used as an alternative to the DIMBOA-based resistance currently used by plant breeders; maize that, unlike maize with DIMBOA-based resistance, is resistant to European corn borer feeding throughout the life of the plant; resistant maize that could be used in rotation with maize that contains genetically engineered insect toxins (for example, *Bacillus thuringiensis* Berliner) to aid in insect resistance management to these toxins; and a novel control solution for maize pests, such as the multiple insect resistance found in Caribbean maize from Antigua (Davis et al. 1988).

The basis of the resistance in the Peruvian maize may be new, or it may be a factor that has already been found to play a secondary role in maize resistance to leaf-feeding European corn borer such as other benzoxalinones (Tipton et al. 1967, Hofman and Hofmanova 1969, Klun et al. 1970) or the silica or lignin content of the leaf material (Rojanardpiched 1983). Maize resistant to European corn borer leaf feeding has been found with low levels of DIMBOA (Sullivan et al. 1974, Scriber et al. 1975, Chiang and Hudon 1976). The basis of the resistance for these low-DIMBOA resistant maize populations has not been described. A knowledge of the basis of resistance for these low-DIMBOA resistant maize accessions would prove useful in developing maize resistant to European corn borer and in maize phylogenetic studies.

Reid et al. (1989) found a positive correlation between susceptibility to European corn borer leaf feeding and altitude while evaluating maize indigenous to Mexico. An association was found between susceptibility to European corn borer leaf feeding susceptibility and altitude in the Peruvian maize we evaluated. Higher-elevation maize was

more susceptible to European corn borer leaf feeding. Why is lower-altitude maize more resistant to European corn borer leaf feeding? Levin and York (1978) have shown that tropical plants have more toxic types of alkaloids occurring in greater amounts than do temperate plants. They hypothesized that these differences result from more intense herbivory in the tropics. When searching for plant germplasm resistant to a particular pest, it may be advisable to begin evaluating sources adapted to lower altitudes and tropical and subtropical conditions, especially if resistance is difficult to find in existing agronomically superior germplasm.

Our oviposition trials indicated that accessions 503796 and 485330 had significantly fewer egg masses than did accessions 485320, 571506, and 515036 (Table 4). Trichome density can alter insect oviposition rates (Norris and Kogan 1980); however, no association was found between trichome density rates and oviposition or between other morphological characteristics and oviposition. The significant differences in ovipositional rates may be caused by undetermined chemical factors or morphological factors. The 2 controls had no significant differences in number of oviposited egg masses. We could not determine the cause for this.

One accession, 571506, had plants with slightly folded or pleated leaves. Many egg masses were deposited on this accession in the pleating of the leaves. This finding may indicate a desirable physical trait for ovipositing European corn borer females. The pleated leaf pattern in accession 571506 was conducive to European corn borer oviposition and may be useful in laboratory rearing this insect by using a pleated or convoluted laying medium (that is, waxed paper).

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